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Studies To Formulate New Media for the Standard Plate Count of Dairy Products

By Leon Buchbinder, Yetta Baris, Edythe Alff, Ernestine Reynolds, and Elizabeth Dillon;* and Vivian Pessin, Louis Pincus, and Aaron Strauss**

The present Standard Methods agar for the bacterial plate count of dairy products, so-called T.G.E.M. agar (Tryptone, 0.5 percent; dextrose, 0.1 percent; beef extract, 0.3 percent; skim milk, 1.0 percent; and agar, 1.5 percent), has been in use for more than 10 years.[1] This medium, called the standard medium in this report, was formulated when attempts were made to obtain a more productive agar than the official standard medium existing at the time. It fulfilled this purpose and was adopted. That it is not an ideal medium is attested by a number of persons who use it routinely.

The disadvantages stem from the presence of two ingredients. One of the ingredients, skim milk, gives rise to several difficulties. In the first place, it takes skill to add skim milk to the medium successfully in the form of dissolved dried powder. Second, after being added to the medium, milk sometimes exhibits a tendency to settle out when the medium is held in a tempering bath prior to pouring. Finally, and perhaps most important, milk induces cloudiness in the agar which, although seeming to make colonies stand out more distinctly at times, actually tends to make counting more difficult. This difficulty is due to the selection which the eye must make between very fine particles of milk and fine colonies. There is little question that, as a rule, colonies on a clear medium are more easily counted. During the experimental work presented here, technicians experienced in the use of the standard medium volunteered the information that the new milkless media studied caused less eyestrain.

A second objection to the present standard medium is the presence of a nonstandardized product, beef extract. The best information

*From the Bureau of Laboratories, and **the Bureau of Records and Statistics, New York City Department of Health. Presented, in part, before the Laboratory Section of the American Public Health Association at the Seventy-sixth Annual Meeting in Boston, Mass., Nov. 9, 1948.

[1] Standard Methods for the Examination of Dairy Products. Ed. 9, American Public Health Association, 1948, p. 93.

that could be obtained concerning this substance is that, regardless of brand name, its composition is unknown. It is a byproduct of meat processing and usually comes from Argentina.

Presented here are studies, made in a laboratory which routinely carries out the standard plate count on dairy products, of a number of media which do not contain the defects mentioned and which yield plate counts equal to or exceeding those of the present standard medium.

Experimental

The general technique used is advocated in the Ninth Edition of Standard Methods for the Examination of Dairy Products. All the new media studied, except two, were prepared directly from ingredients in this laboratory. They were compared with the standard medium, prepared in a similar manner, and in some instances with each other. Pasteurized milk was the product most frequently used for the tests, although raw milk and pasteurized cream also were studied.

Dilutions of $1/100$ and $1/1000$ were used for pasteurized milk, and $1/1000$ and $1/10000$ for raw milk and pasteurized cream. Duplicate plates in each of the two dilutions were prepared for both the test and standard media. When several test media were studied simultaneously, four plates were made with each medium tested. Only one dilution was counted for record purposes; that which showed counts under 300 was preferred. The mean of the duplicate plates of the test medium in the dilution used and the corresponding mean of the standard medium are referred to as a "paired count."

Statistical Method

When comparing two series of observations in which each observation of one series is positively correlated with an observation in the other, it is more efficient to work with the differences of the paired observations than to treat the two series as independent. This is so because the first method will give results which will vary less than those of the second if the experiment is replicated.

It is also demonstrable that in certain kinds of problems involving bacteriological counts the use of logarithms will result in more reliable statistics. For example, a series of counts taken from different samples will usually not be normally distributed, while the distribution of the logarithms of such counts will be approximately normal. In such cases, where normal curve theory is to be applied, the correct procedure is to convert the counts to logarithms before performing the computations.

It was determined, for each test medium, the probability that the average difference between the pairs of logarithms (test medium and

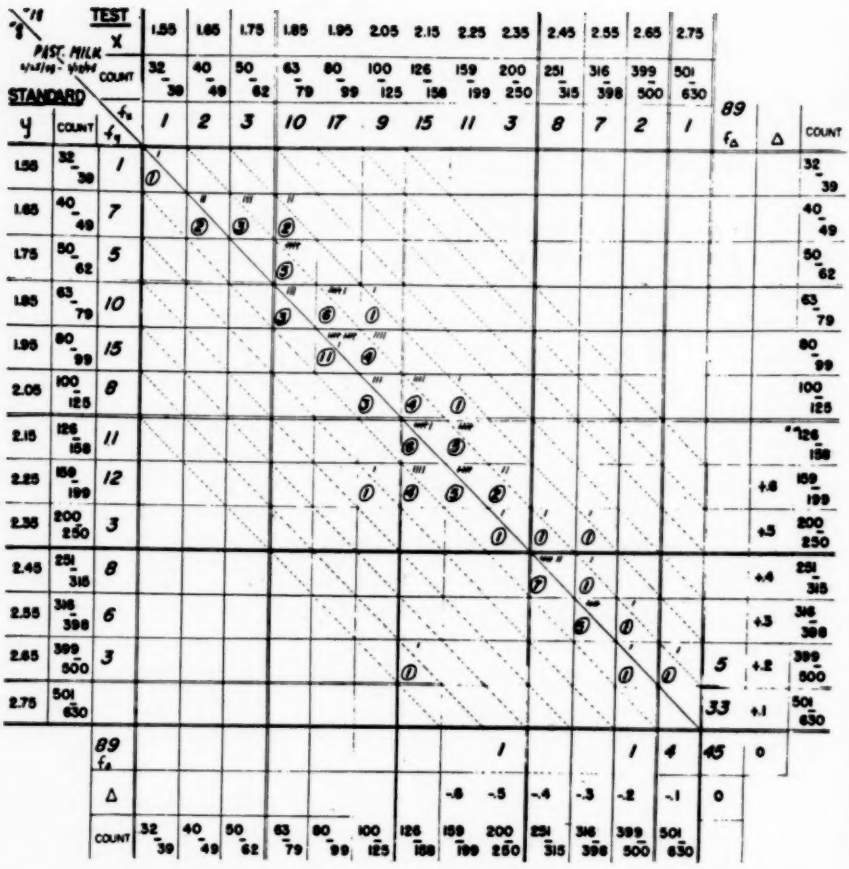
standard medium counts for the same sample) could have been exceeded by chance alone, if there were actually no difference between the test and standard media. When this probability was less than 1 chance in 20 ($\bar{\Delta}/\sigma_{\bar{\Delta}} \geq 1.96$), the difference was said to be statistically significant. All the differences listed in the tables, except those marked with an asterisk, are statistically significant. The last column in the tables gives the range within which the true percent difference lies approximately 95 percent of the time.

When the volume of data is relatively small, the labor involved in conversion to logarithms is not prohibitive. However, when the data are extensive and computing equipment limited, practical considerations may present a serious, and sometimes insuperable, obstacle to the use of logarithms.[2] The procedure used in this analysis, although somewhat less accurate than the use of ungrouped logarithms, is not biased; and it eliminates the drudgery of conversion to logarithms. Essentially, it consists of the use of a correlation table, with the class limits of counts chosen so that the corresponding class intervals of the logarithms of the counts are equal in width. Although some information is lost when wide class intervals are used instead of exact values, the loss is not important when one is concerned with the exploration of voluminous data to detect major differences.

Since this procedure, or adaptations of it, may be of use elsewhere, it is described in some detail. The type of work sheet used is illustrated in the chart. The numbers and symbols in roman type are printed on the work sheet. The tally marks and the numbers in italics were recorded while working on the results of one series of counts with the standard medium (No. 8) and test medium (No. 18), using pasteurized milk. The rows and columns on the original work sheet which were not used for this sample are omitted from the illustration.

The column and row headed "Count" refer to the observed counts of the standard and test media, respectively. The class limits of the count intervals are chosen so that their logarithms are 0.1 unit apart. Thus the logarithms of 32 to 39 fall in the class interval 1.50–1.59, the logarithms of 40 to 49 fall in the class interval 1.60–1.69, etc. The midpoints of the logarithm classes are given in the first column and in the first row (y and x).

[2] The conflict that sometimes arises between theoretical requirements and practical possibilities is ably expressed by W. G. Cochran in his monograph, The Present Status of Biometry: "In view of the growing complexity of statistical techniques, a trend which is unlikely to be reversed, more attention might profitably be given to simpler methods whose efficiency is satisfactory if not the maximum attainable. It is easy for the statistician, who may use his method only in the preparation of an example to be included in a publication, to persuade himself that the method is relatively simple, but his views might change if he were forced to apply it continually with poor computing equipment . . . It is interesting to note that in the field of quality control a deliberate campaign has been carried on to keep the techniques very elementary, even at a loss both in efficiency and in flexibility. While this has sometimes led to teaching of rules of procedure rather than principles, it has contributed to the rapid dissemination of the methods and is evidence of the sound attitude that the methods are a means and not an end."



Δ	f_a	Δf_a	$\Delta^2 f_a$
-.5	1	-.5	.25
-.2	1	-.2	.04
-.1	4	-.4	.04
0	45	0	0
.1	33	3.3	.33
.2	5	1.0	.20
	89	3.2	.86

$N = \Sigma f_a = 89$

$\bar{\Delta} = \frac{\Sigma \Delta f}{N} = \frac{3.2}{89} = .036$

$\sigma_{\bar{\Delta}} = \frac{\sqrt{\Sigma \Delta^2 f - \Delta \Sigma \Delta f}}{N}$

$\sigma_{\bar{\Delta}} = \frac{\sqrt{.86 - (.036)(3.2)}}{89}$

$\sigma_{\bar{\Delta}} = .0097$

$\frac{\bar{\Delta}}{\sigma_{\bar{\Delta}}} = \frac{.036}{.0097} = 3.7$

$\bar{y} = \frac{\Sigma y f_a}{N} = \frac{18655}{89} = 2.096$ $\quad$ antilog $\bar{y} = 125$ - G.M.$_y$

$\bar{x} = \frac{\Sigma x f_a}{N} = \frac{18975}{89} = 2.132$ $\quad$ antilog $\bar{x} = 136$ - G.M.$_x$

$2\sigma_{\bar{\Delta}} = 2(.0097) = .0194$ $\quad$ antilog $(2\sigma_{\bar{\Delta}}) = 1.05$

Ratio Of Test Medium To Standard Medium

Average $= \frac{136}{125} \times 100 = 109$

Lower Limit $= \frac{109}{1.05} = 104$ $\quad$ Upper Limit $= 109(1.05) = 114$

Work sheet for testing significance of mean difference of logarithms of paired counts. Short method.

The sheet is used first to tally the counts. A count of 488 on the standard medium and of 138 on the test medium is tallied in the box on the 399–500 row and in the 126–158 column. The remaining pairs of counts are similarly tallied. The number of tallies in each box is written down and circled. By adding across rows (sums in the f_y

column), and down columns (sums in the f_x row), frequency distributions of the counts and of their logarithms are obtained. These distributions are used as a check of the tallying and of the normality of the data, and also for calculating the means of the two media. By adding along the diagonal lines and entering the sums in the f_Δ column and row, a frequency distribution of the differences of logarithms is obtained. This distribution is copied into the table at the lower left-hand corner, and calculations are completed as illustrated. If the ratio of the mean difference of the logarithms to its standard error ($\bar{\Delta}/\sigma_{\bar{\Delta}}$) is 1.96 or more, the difference between the two media may be considered significant.

To summarize, this procedure substitutes tallying and addition for (1) conversion to logarithms, (2) finding the difference of the pairs, and (3) formation of a frequency distribution. That both methods yield approximately the same results was shown by taking the data for a few test media and testing by the long method without grouping. The results were practically the same by both methods of calculation.

The work sheet was also used in the laboratory to check the results of test media after relatively few (15 or 20) counts were made. By tallying the results, and adding to form the frequency distribution, an estimate of the mean difference of the logarithms was obtained. The direction and size of the difference were two of the factors considered in deciding whether work on the medium was to be continued. Where the decision was affirmative, reference was made to a graph showing the estimated number of paired counts needed, with the given mean difference, to demonstrate a significant difference. This estimated number was then used as a guide to the amount of additional work needed on the test media.

Results

The first media studied were unsatisfactory in that they were not as productive as the standard medium. These unsuccessful media comprised individual peptones, such as Trypticase, Tryptone, and Proteose Peptone No. 3, either alone or in combination, usually with dextrose and occasionally with sodium succinate and sodium citrate.

Media Containing Difco Yeast Extract, a Peptone, and Dextrose as Essential Ingredients

The first successful medium found was No. 16, listed in table 1, which comprised Difco Yeast Extract, 1.5 percent; Difco Proteose Peptone No. 3, 0.5 percent; dextrose, 0.1 percent; sodium succinate, 0.2 percent; and agar, 1.5 percent. Pasteurized milk, raw milk, and pasteurized cream, when tested with this medium, yielded counts which were significantly higher than those found with the control standard medium. As seen in the table the difference was about 10

Table 1. *Productivity of various test media compared with the standard medium (T.G.M.)*

[Test media with Difco Yeast Extract, a peptone, and dextrose as essential ingredients]

Test medium No.	Composition of test media (percent)					Product	Number samples	Geometric means				Confidence limits of percent difference (∓1.96σ)
	Difco Yeast Extract	Difco Proteose Peptone No. 3	Dextrose	Sodium succinate	Agar			Test medium	Standard medium (T.G.M.)	Percent difference (standard=100) Pos.	Percent difference (standard=100) Neg.	
16	1.5	0.5	0.1	0.2	1.5	Past. milk	181	166	151	10		+7, +13
						Raw milk	63	258	231	12		+7, +17
						Past. cream	35	104	93	12		+6, +17
	Changes in proportion of ingredients of medium No. 16											
18	1.5	0.5	0.1		1.5	Past. milk	164	155	141	10		+7, +13
						Raw milk	132	212	196	8		+6, +11
						Past. cream	77	95	88	8		+4, +12
18C	(1)	(1)	(1)	(1)	(1)	Past. milk	42	172	167	3		0+, + 6
						Raw milk	66	156	149	5		+1, + 8
						Past. cream	59	98	90	9		+5, +13
22	1.0	.5	.1	.2	1.5	Past. milk	44	294	272	8		+4, +13
23	1.0	.5	.1		1.5	Past. milk	91	217	212	*2		−1, + 6
17	2.0		.1	.2	1.5	Past. milk	76	153	163		6	−11, − 1
25	1.0	.5			1.5	Past. milk	33	173	209		17	−25, − 9

*Differences marked with an asterisk are statistically not significant (P>0.05). All other differences are significant.
1 Same as No. 18 with addition of cover agar.

percent when based on the geometric means of the counts with each of the two media.

Changes in Proportion of Ingredients

It is a matter of debate whether a new standard medium should be more productive than the existing standard medium, since greater productivity might cause administrative difficulties. Attempts were made, therefore, to reduce the productivity of No. 16 by manipulating the quantities of ingredients or by omitting selected ingredients. Another consideration was the fact that No. 16 tended to promote surface growth which, at times, resulted in the presence of large spreader type colonies which interfered with counting.

The first alternative medium No. 18 which did not contain sodium succinate, was slightly less productive than No. 16. Medium No. 18C, which was the same as No. 18 except that a cover of neutral agar was placed over the solidified medium in order to reduce surface spreading, showed, as might have been expected, a still greater reduction when compared with the standard medium. Medium No. 22 (No. 16 modified by reducing the concentration of yeast extract by one-third) likewise yielded a smaller percent difference from the standard medium than did No. 16. Medium No. 23 (No. 22 with sodium succinate omitted) showed a still greater reduction in the percent of difference. This medium yielded only about 2 percent more colonies than did the standard medium by the method of comparison used.

When medium No. 22 was altered by omitting Proteose Peptone No. 3 and doubling the quantity of yeast extract (No. 17), and when medium No. 22 was changed by omitting dextrose and sodium succinate (No. 25), it was found that the productivity of both was significantly less than that of the present standard medium. It may be concluded, therefore, that a medium containing 1.0 percent Difco Yeast Extract, 0.5 percent Difco Proteose Peptone No. 3, and 0.1 percent dextrose (No. 23) might serve as a satisfactory substitute for the present standard medium. This medium is clear, has a slightly yellow tinge caused by the yeast extract, and does not seem to produce more surface spreaders than does the standard medium (table 1).

Substitution of Other Peptones

The feasibility of substituting other peptones for Proteose Peptone No. 3 (medium No. 23) was next investigated. The peptones tested were Difco Tryptone, Baltimore Biological Laboratory Trypticase, and B.B.L. Polypeptone (Nos. 29, 30, 24). It was found that any of these peptones could be substituted and that the medium containing Polypeptone yielded the greatest number of colonies when pasteurized milk was the product tested (table 2). It is of interest that with

Table 2. *Productivity of various test media compared with the standard medium (T.G.M.)*

[Test media with Difco Yeast Extract, a peptone, and dextrose as essential ingredients]

Test medium No.	Composition of test media (percent)							Product	Number samples	Geometric means				Confidence limits of percent difference (∓1.96σ)
	Difco Yeast Extract	Difco Proteose Peptone No. 3	B.B.L. Polypeptone	Difco Tryptone	B.B.L. Trypticase	Dextrose	Agar			Test medium	Standard medium (T.G.M.)	Percent difference (standard=100) Pos.	Percent difference (standard=100) Neg.	
	Substitution of other peptones in medium No. 23													
23	1.0	0.5				0.1	1.5	Past. milk	91	217	212	*2		− 1, + 6
29	1.0			0.5		.1	1.5	Past. milk	58	200	189	6		+ 1, +11
30	1.0				0.5	.1	1.5	Past. milk	168	167	160	4		+ 2, + 7
								Raw milk	51	182	175	4		+ 2, + 6
24	1.0		0.5			.1	1.5	Past. milk	207	226	206	10		+ 7, +12
								Raw milk	17	246	234	5		+ 4, + 6
	Reduced quantity of B.B.L. Polypeptone in medium No. 31													
31	0.8		0.5			0.1	1.5	Past. milk	158	301	265	14		+10, +17
36	.8		.4			.1	1.5	Past. milk	51	317	274	16		+11, +21
37	.8		.3			.1	1.5	Past. milk	51	303	274	11		+ 6, +15
38	.8		.2			.1	1.5	Past. milk	83	299	287	4		0+, + 8

*Differences marked with an asterisk are statistically not significant (P>0.05). All other differences are significant.

pasteurized milk the Polypeptone medium (No. 24) produced significantly higher counts than did the Proteose Peptone No. 3 medium (No. 23) when these were compared directly (66 samples).

Reduced Quantity of B.B.L. Polypeptone

The effect of varying the amount of Polypeptone used was next studied. The four media studied contained Difco Yeast Extract, 0.8 percent; dextrose, 0.1 percent; agar, 1.5 percent; and quantities of Polypeptone which decreased decimally from 0.5 percent (No. 31) to 0.2 percent (No. 38). The quantity of Difco Yeast Extract used in these media was reduced from 1.0 to 0.8 percent because of experiments described below. Nos. 31, 36, 37, and 38 yielded significantly greater numbers of colonies than did the standard medium. However, when No. 38, containing 0.2 percent Polypeptone, was compared directly with the other three test media, it was found to yield lower counts than all of them. This difference was significant for both Nos. 31 and 36 (table 2).

Substitution of Other Yeast Extracts

Attempts were made to substitute other yeast products for Difco Yeast in No. 18. Fleischmann's Yeast Autolysate (No. 26), B.B.L. Yeast Extract (No. 27), and Basamin-Busch Yeast Extract of Anheuser-Busch (No. 28) were each studied separately; the other nutriments in each of the media being Proteose Peptone No. 3, 0.5 percent; and dextrose, 0.1 percent. All of these latter media were significantly inferior to the standard medium (table 3).

Reduced Quantity of Difco Yeast Extract

Studies were made to determine to what extent the amount of Difco Yeast Extract could be reduced and still produce a satisfactory medium with Polypeptone, 0.5 percent; and dextrose, 0.1 percent. Four media were compared with the standard medium (No. 24; No. 31, containing 0.8 percent yeast extract; No. 32, containing 0.6 percent yeast extract; No. 33, containing 0.4 percent yeast extract). All of these media might be satisfactorily substituted for the standard medium with the exception of No. 33 (0.4 percent yeast extract) which yielded about 5 percent fewer colonies than did the standard medium. This difference, however, is not statistically significant. The first three test media each yielded counts significantly greater than those of No. 33 (table 3).

Substitution of Tryptone and Reduced Quantity of Difco Yeast Extract in Medium No. 24

Tests were carried out to ascertain whether substitution of Difco Tryptone for Polypeptone in media with substantially reduced quan-

Table 3. *Productivity of various test media compared with the standard medium (T.G.M.)*

[Test media with Difco Yeast Extract, a peptone, and dextrose as essential ingredients]

Test medium No.	Composition of test media (percent)									Product	Number samples	Geometric means				Confidence limits of percent difference (∓1.96σ)
	Difco Yeast Extract	Fleischmann's Yeast Autolysate	B.B.L. Yeast Extract	Anheuser-Busch Yeast Extract (Basamin-Busch)	Protose Peptone No. 3	B.B.L. Polypeptone	Difco Tryptone	Dextrose	Agar			Test medium	Standard medium (T.G.M.)	Percent difference (standard=100) Pos.	Percent difference (standard=100) Neg.	
	Substitution of other yeast extracts for Difco Yeast Extract in medium No. 18															
18	1.5				0.5			0.1	1.5	Past. milk	164	155	141	10		+7, +13
26		1.5			.5			.1	1.5	Past. milk	23	150	247		39	−47, −31
27			1.5		.5			.1	1.5	Past. milk	66	185	205		10	−15, −4
28				1.5	.5			.1	1.5	Past. milk	30	148	200		26	−32, −20
	Reduced of quantity of Difco Yeast Extract in medium No. 24															
24	1.0					0.5		0.1	1.5	Past. milk	207	226	206	10		+7, +12
31	.8					.5		.1	1.5	Past. milk	158	301	265	14		+10, +17
32	.6					.5		.1	1.5	Past. milk	87	274	244	12		+8, +17
33	.4					.5		.1	1.5	Past. milk	87	233	244		*5	−9, 0+
	Substitution of Tryptone and reduced quantity of Difco Yeast Extract in medium No. 24															
44	0.5						0.5	0.1	1.5	Past. milk	130	129	126	*2		−4, +9
45	.35						.5	.1	1.5	Past. milk	97	123	127		*3	−9, +3

*Differences marked with an asterisk are statistically not significant (P>0.05). All other differences are significant.

tity of Difco Yeast Extract would affect the results. This substitution, as is seen in the table (Nos. 44, 45), produced no substantial change (table 3).

Media Composed of Baltimore Biological Laboratory Products[3]

The first medium (No. 19) studied contained B.B.L. Yeast Extract, 1.5 percent; B.B.L. Phytone, a plant peptone, 0.5 percent; dextrose, 0.1 percent; and sodium succinate, 0.2 percent. This medium was significantly less productive than the standard medium when tested with pasteurized milk (table 4). Another medium (No. 20) containing Polypeptone, 1.5 percent; Phytone, 0.5 percent; dextrose, 0.1 percent; and sodium succinate, 0.2 percent, was tested with both pasteurized and raw milk. The data in the table indicate that only the raw milk samples yielded significantly higher counts. An additional medium tested (No. 21), consisted of Trypticase, 1.5 percent; Phytone, 0.5 percent; dextrose, 0.5 percent; sodium citrate, 0.1 percent; and sodium chloride, 0.4 percent. This product, which was supplied by the Baltimore Biological Laboratory in powdered form and was one of two prepared media studied, was tested with pasteurized milk. The counts were significantly lower than those with the standard medium.

Substitution of Phytone for Yeast in Medium No. 24

A fourth medium studied (No. 39) was modeled after No. 24 with the substitution of Phytone, 1.0 percent, for Difco Yeast, 1.0 percent. The products tested were pasteurized milk and pasteurized cream. The counts with this medium were significantly greater than those with the standard medium (table 4).

Changes in Ingredient Proportions in Medium No. 39

Reduction of the quantity of Polypeptone in medium No. 39 from 0.5 to 0.4 or 0.3 percent (Nos. 40, 41) reduced the percent difference in counts to zero; further reduction in the quantity of this peptone (No. 42) resulted in lower counts than those found with the standard medium. The effect of reduction in quantity of Phytone (Nos. 43, 46) could be partially compensated for by increasing the amount of Polypeptone (No. 47).

No increase in count resulted from the addition of sodium sulfite and cystine in very small quantities to a dehydrated batch of medium No. 39 prepared by the Baltimore Biological Laboratory (No. 49) (table 4).

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[3] According to the Baltimore Biological Laboratory:
Phytone is a papaic digest of soya meal. Trypticase is a peptone derived from casein by pancreatic digestion. Polypeptone is made up of equal parts of Trypticase and Thiotone. Thiotone is a peptic hydrolysate of animal tissues.

Table 4. *Productivity of various test media compared with the standard medium (T.G.M.)*

[Test media with B.B.L. products]

Test medium No.	Composition of test media (percent)											Product	Number samples	Geometric means				Confidence limits of percent difference (∓1.96σ)
	B.B.L. Yeast Extract	B.B.L. Phytone	B.B.L. Polypeptone	B.B.L. Trypticase	Dextrose	Sodium succinate	Sodium citrate	Sodium chloride	Sodium sulfite	Cystine	Agar			Test medium	Standard medium (T.G.M.)	Percent difference (standard=100)		
																Pos.	Neg.	
19	1.5	0.5			0.1	0.2					1.5	Past. milk	44	246	272		10	−14, −5
20		.5	1.5		.1	.2					1.5	Past. milk	141	145	145	*0		−3, +3
												Raw milk	59	174	161	8		+5, +11
21		.5		1.5	.5		0.1	0.4			1.5	Past. milk	44	223	272		18	−23, −13
	Substitution of Phytone for yeast in medium No. 24																	
39		1.0	0.5		0.1						1.5	Past. milk	145	145	137	6		0+, +12
												Past. cream	95	99	91	9		+5, +13
	Changes in proportion of ingredients in medium No. 39																	
40		1.0	0.4		0.1						1.5	Past. milk	81	120	120		*0	−8, +8
41		1.0	.3		.1						1.5	Past. milk	81	120	120		*0	−8, +8
42		1.0	.2		.1						1.5	Past. milk	81	110	120		*8	−18, +2
43		.5	.5		.1						1.5	Past. milk	71	114	133		14	−21, −7
46		.35	.5		.1						1.5	Past. milk	38	112	143		22	−32, −10
47		.35	1.0		.1						1.5	Past. milk	59	115	118		*3	−9, +5
49		1.0	.5		.1				0.04	0.04	1.5	Past. milk	173	129	127	*2		−2, +6

*Differences marked with an asterisk are statistically not significant (P>0.05). All other differences are significant.

Discussion

The necessity for adequate statistical evaluation of data obtained in quantitative studies of this type cannot be too strongly emphasized. A number of previous studies in this very field have been marred by cumbersome, and sometimes inadequate or incorrect, treatment of data. The particular method used here, in addition to being statistically valid, included a "short cut" which saves much time in analysis. This "short cut," the use of class intervals of counts which correspond to equal class intervals of logarithms, may be adapted to other problems involving transformation of numbers to logarithms. Finally, the method provides a means whereby, in the absence of other governing considerations, it can readily be determined when to continue or discontinue a given series of tests and how to estimate in advance the number of tests needed to arrive at a statistically significant conclusion.

Apparently, an adequate medium for the standard plate count of dairy products must contain no less than three different nutriments. According to the data presented, these are: a yeast extract (Difco) or a plant peptone (B.B.L. Phytone); an animal peptone (any one of several); and carbohydrate (dextrose).

Despite the fact that milk product samples tested contained diverse species and numbers of bacteria, manipulation of the quantities and kinds of ingredients in the media studied was found to have predictable quantitative effects. Although such a result might be expected in pure culture studies, it was somewhat surprising in this instance. A partial explanation lies in the use in the present study of the "paired count," i. e., a direct comparison of the results obtained on both the test and standard media for each sample of milk product studied.

If and when the Standard Methods Committee selects a new medium or media for the plate count, it can determine the productivity desired and establish the quantities of ingredients which must be used to attain it.

Summary

1. Reasons for the desirability of formulating a new standard medium for the plate count of dairy products are given.

2. Studies are described in which several satisfactory media were found.

3. One type of medium contains Difco Yeast Extract, dextrose, and a peptone or peptone mixture as essential ingredients. Yeast extracts produced by three other companies were found to be unsatisfactory for this purpose. Each of four peptones tested was found to be satisfactory. They were B.B.L. Polypeptone and Trypticase, and Difco Tryptone and Proteose Peptone No. 3. The medium con-

taining B.B.L. Polypeptone was found to produce significantly higher counts than did the one containing Proteose Peptone No. 3.

4. Another apparently satisfactory type of medium contains the plant peptone, B.B.L. Phytone, an animal peptone, and dextrose as essential ingredients.

5. It is shown that manipulation of the amounts of ingredients or substitution or omission of certain ingredients has predictable quantitative effects on the plate count.

6. A short method for testing the statistical significance of the mean difference of logarithms of paired counts is described and illustrated.

Incidence and Distribution of *Brucella abortus* in Slaughtered Bang's Reactor Cattle

By Norman B. McCullough, Ph.D., M.D.,*† C. Wesley Eisele, M.D.,* and Anne F. Byrne*

In considering the occurrence of brucellosis in packing-house workers, the Bang's reactor cow has received inadequate attention. It has been frequently stated that in reactor cattle the organism is found mainly in the uterus and the udder; hence, the remainder of the carcass provides little or no exposure hazard to the worker. The prevalence of this view is somewhat surprising. It has long been known that early in the course of the disease the organism may be widely disseminated (*1*). *Brucella* has been isolated from the blood stream of cattle in a number of studies (*2*, *3*, *4*). Recently, Manthie and Carter performed blood cultures on artificially infected cattle (*5*). Of 270 cows, *Brucella abortus* was recovered from the blood of 172 animals. In 18 animals studied over a prolonged period, bloodstream infection persisted in over 80 percent for 5 months and in one animal for almost 2 years. These studies suggest an exposure hazard greater than that usually recognized.

More definitive information seemed to be needed on the extent of infection in a substantial number of animals actually sent for slaughter.

In this study the carcasses of 100 Bang's reactor cattle were examined.

Materials and Methods

To conduct the study, arrangements were made with one of the large packing plants in the Chicago area. Over a period of 6 months, 100 Bang's reactor cattle sent for slaughter were examined. They were unselected except for the known positive Bang's reaction. They were all of dairy breeds. The series comprised 88 cows, 9 heifers, 2 calves, and 1 bull. They were derived from herds in five Midwestern States.

During bleeding, a blood sample was obtained for an agglutination test. At various stages of the dressing process, tissues were obtained for culture. Accessible lymph nodes in widespread areas of the carcass, tonsil, and sections of liver, spleen, uterus, and membranes and fetus, if present, were obtained. Under plant conditions, the

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mammary gland was not accessible to us. Samples from all selected areas could not always be obtained. Nor was it possible to obtain tissues in an entirely aseptic manner. However, separate sterile instruments were used for obtaining each specimen which was then placed in a separate sterile screw-capped glass jar. Upon return to the laboratory, the samples were promptly cultured. Each lymph node or other tissue was trimmed of fat, seared in a flame, sectioned, and the cut surface serrated and streaked directly on the surface of Trypticase soy agar medium. Sterile instruments were used throughout. Plates were inoculated in duplicate and incubated at 37° C. in an atmosphere of 10 percent added CO_2.

In spite of the strict technique employed, occasional plates were overgrown with extraneous organisms and were discarded. Such tissue specimens are omitted from the tabulation of data.

Upon isolation of *Brucella*, cultures were identified by the usual methods, and the species was established by determination of CO_2 requirement, H_2S production, growth on differential dye plates, and the use of specific absorbed typing sera. Each strain was inoculated into guinea pigs. Four weeks later the blood was tested for the presence of agglutinins; the guinea pigs were sacrificed, and cultures were made of the tissues.

Using the standardized antigen regularly employed in our laboratory (*6*) with incubation at 37° C. for 48 hours, we determined the agglutination titer by means of the standard test-tube agglutination test.

Results

Of the 100 cattle examined, *Br. abortus* was recovered from one or more areas of the carcass in 42 animals. In 29, the organism was obtained from locations other than the uterus or supramammary lymph nodes. Table 1 presents the detailed results from the tissue cultures in these 42 animals. In ten of these, *Br. abortus* was isolated from numerous locations in widespread areas of the carcass.

All isolations of *Brucella* adhered to the characteristics of the species and typed readily by the methods mentioned. They all required CO_2 for initial growth. With one exception, all proved pathogenic for the guinea pig. The organism isolated from cow No. 15 failed to produce agglutinins in the guinea pig upon repeated trials and was not recovered from post-mortem cultures.

Table 2 presents the composite results of all isolations in the series tabulated according to frequency of isolation from particular tissues. Table 3 presents the distribution of *Br. abortus* in the tissues of the 42 culturally proved cases of Bang's disease. With the exception of the external iliac nodes, of which only seven specimens were obtained, and the sternal and vertebral marrow, every site cultured yielded

one or more isolations. The highest incidence of recovery was in cultures from the supramammary lymph nodes. Of 99 such specimens examined, 28.3 percent contained *Br. abortus*. The next highest incidence was in the uterus, with 15.6 percent. Although the uterus and the supramammary lymph nodes are most regularly involved, the occurrence of infection in widely scattered areas of the carcass is shown by these data.

There was some relationship between the agglutination titer and

Table 1. *Recovery of* **Brucella abortus** *from tissues of 42 Bang's reactor cattle*

Lymph node or organ	Animal No.													
	3	4	9	10	11	12	15	22	28	30	32	34	42	43°
Atlantal		+						−						
Parotid	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Submaxillary	−	−	+	−	−	−	−	−	+	+	−	−	−	−
Tonsil	−	+	−	−	−	−	−	−	+	−	−	−	−	−
Retropharyngeal	−	+	−	−	−	−	−	−	+	−	−	−	−	−
Prescapular			−	−	−	−	−	−	+	−	−	−	−	+
Mediastinal	−	−	−	−	−	−	−	−	+	+	−	−	−	+
Bronchial	−	−	−	−	−	−	−	−	+	+	−	−	−	−
Splenic	−	−	−	−	−	+	−	−	+	−	−	−	−	−
Portal	−	−	−	−	+	−	−	−	+	−	−	−	−	−
Ileocecal	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Mesenteric	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Supramammary	+		−	+	+	+	−	+	+	−	−	−	+	+
Prefemoral			−	−	+	−	+	−	−	−	−	−	−	−
Internal iliac	−	−	−	−	−	−	−	−	+	+	−	−	−	−
External iliac				−	−	−								
Spleen	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Liver		−	−	−	−	−	−	−	−	−	−	−	−	−
Uterus	+	+	+	+	−	+	−	−	−	−	+	+	−	−
Placenta	+		+	+	+			−					−	
Membranes and/or fetus														
Sternal marrow								−	−	−	−	−	−	−
Vertebral marrow								−	−	−	−	−	−	−
	P	N	P	P	P	RP	N	P	N	P	N	RP	P	N

Lymph node or organ	Animal No.													
	44°	51	53	55	56	57	58	63	64	70	75	76	77	80
Atlantal														
Parotid	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Submaxillary	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tonsil	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Retropharyngeal	−	−	−	−	+	−	−	−	−	+	−	−	−	−
Prescapular	−	−	+	−	−	−	−	+	−	+	−	−	−	+
Mediastinal	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bronchial	+	−	−	−	−	−	−	−	−	−	−	−	−	+
Splenic	−	−	−	+	−	−	−	−	−	+	−	−	−	−
Portal	−	+	−	−	−	−	−	−	−	+	−	−	−	+
Ileocecal	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Mesenteric	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Supramammary	+	−	+	+	+	+	+	+	+	+	+	+	+	+
Prefemoral	−	−	+	−	−	−	−	−	−	−	−	−	−	+
Internal iliac	−	−	−	+	+	−	−	−	−	−	−	−	−	+
External iliac														
Spleen	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Liver	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Uterus	−	+		+	+	−	−	+	−	−	−	−	−	−
Placenta						−					−	−	−	−
Membranes and/or fetus						−					−	−	−	−
Sternal marrow	−	−	−	−	−	−				−				
Vertebral marrow	−	−	−	−	−									
	N	N	N	N	N	P	N	N	N	N	P	P	P	P

+ = positive; − = negative; ° = calf; P = pregnant; N = nonpregnant; RP = recent parturition; *Positive culture from membranes.

Table 1. *Recovery of* Brucella abortus *from tissues of 42 Bang's reactor cattle*—Con.

Lymph node or organ	Animal No													
	81	82	83	84	88	89	90	92	93	94	96	97	98	100
Atlantal														
Parotid	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Submaxillary	−	−	−	−	−	−	−	−	−	−	−	−	+	−
Tonsil	−	−	−	−	−	−	−	+	−	−	−	+	−	−
Retropharyngeal	−	−	−	−	−	−	−	−	+	+	+	−	+	−
Prescapular	−	−	+	+	+	−	−	−	−	−	−	−	−	−
Mediastinal	−	+	−	−	−	−		−	−	−	−	−	+	−
Bronchial	+	+	+	−	−	−	+	−	−	−	−	−	−	−
Splenic	−	+	−	−	−		−	−	−	−	−	−	+	
Portal	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Ileocecal	+	+	−	+	−	−	−	−	−	−	−	−	−	
Mesenteric	−	+	−	+	−	−	−	−	−	−	−	−	−	−
Supramammary	−	−	+	+	+	+	−	−	−	−	+	−	+	+
Prefemoral	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Internal iliac	−	−	+	+	+	−	−	−	−	−	−	−	+	+
External iliac														
Spleen	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Liver	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Uterus	−	+	+	−	+		−	−	−	−	+	−	−	−
Placenta		−	+				−		−			−	−	−
Membranes and/or fetus		−	•+						−			−	−	−
Sternal marrow														
Vertebral marrow														
	N	P	P	N	N	N	P	RP	P	N	N	P	P	P

+=positive; −=negative; P=pregnant; N=nonpregnant; RP=recent parturition; •Positive culture from membranes.

Table 2. *Frequency of isolation of* Brucella abortus *from certain tissues of 100 Bang's reactor cattle*

Lymph node or organ	Number cultured	Number positive	Percent positive	Lymph node or organ	Number cultured	Number positive	Percent positive
Atlantal	8	1	12.5	Supramammary	99	28	28.3
Parotid	100	1	1.0	Prefemoral	95	7	7.3
Submaxillary	100	4	4.0	Internal iliac	99	10	10.1
Tonsil	99	4	4.0	External iliac	7	0	.0
Retropharyngeal	100	8	8.0	Spleen	99	2	2.0
Prescapular	95	9	9.5	Liver	95	3	3.1
Mediastinal	93	5	5.4	Uterus	96	15	15.6
Bronchial	95	8	8.4	Placenta	48	5	10.4
Splenic	97	6	6.2	Membranes and/or fetus	28	•1	3.6
Portal	97	6	6.2	Sternal marrow	49	0	.0
Ileocecal	97	5	5.1	Vertebral marrow	40	0	.0
Mesenteric	100	3	3.0				

•Positive culture from membranes.

Table 3. *Distribution of* Brucella abortus *in tissues of 42 cattle with culturally proved Bang's disease*

Lymph node or organ	Number cultured	Number positive	Percent positive	Lymph node or organ	Number cultured	Number positive	Percent positive
Atlantal	2	1		Supramammary	41	28	68.3
Parotid	42	1	2.4	Prefemoral	40	7	17.5
Submaxillary	42	4	9.5	Internal iliac	42	10	23.8
Tonsil	42	4	9.5	External iliac	3	0	
Retropharyngeal	42	8	19.0	Spleen	42	2	4.8
Prescapular	40	9	22.5	Liver	41	3	7.3
Mediastinal	41	5	12.2	Uterus	40	15	37.5
Bronchial	42	8	19.0	Placenta	18	5	27.8
Splenic	40	6	15.0	Membranes and/or fetus	11	•1	9.1
Portal	42	6	14.3	Sternal marrow	14	0	.0
Ileocecal	41	5	12.2	Vertebral marrow	12	0	.0
Mesenteric	42	3	7.1				

•Positive culture from membranes.

the recovery of *Brucella*. All of the animals examined proved to be positive reactors with 80 percent giving titers of 1:640 or higher. Recoveries were made more consistently in the animals with high titers; 92 percent of the culturally positive animals had titers of 1:640 or higher. Titers of 1:2560 or higher occurred twice as frequently in the animals from which *Brucella* was recovered as in the culturally negative animals. An analysis of the relationship of the agglutination titer to pregnancy or nonpregnancy revealed no significant difference. Likewise, the incidence of recovery of the organism was not significantly different in these two groups.

Comment

The failure to recover *Brucella* in 58 of the 100 animals is not surprising. It is probable that a number of these cattle were sent for slaughter because they were no longer profitable and may represent long-term infections. In such cases, infection may be limited to a few localized areas. That one may fail to include such a localized infected area in a cultural sampling of the carcass may be expected. Even a known infected lymph node will not yield the organism unless the infected portion of the node is sectioned and streaked upon the plate. It is interesting to speculate whether some of the animals studied may have been reactors as a result of vaccination.

The predominance of the recoveries of *Brucella* from the uterus and the supramammary lymph nodes is in accord with previous experience.

The isolation of *Brucella abortus* from numerous sites in a significant number of the animals examined emphasizes the potential exposure to *Brucella* encountered by personnel engaged in processing the carcasses of Bang's reactor cattle.

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Charcoal Agar Culture Medium for Preparing *Hemophilus pertussis* Vaccine

By H. M. Powell, C. G. Culbertson, and P. W. Ensminger*

In 1947, Powell, while on a visit to the Lister Institute, talked over briefly with Sir Paul Fildes some of the prevailing difficulties of large scale culturing of *Hemophilus pertussis*. Use of charcoal in place of the blood in the culture medium was suggested by Fildes. At about this time, Pollock (*1*) reported from the laboratories of the Lister Institute that charcoal could replace blood in pertussis culture media, and that the active fraction of blood was found to be the albumin fraction which neutralized the toxic action of fatty acids.

During the past year we have tried charcoal agar for growing mass cultures of *H. pertussis* to produce vaccine. Our use of charcoal agar followed preliminary experiments in making charcoal broth by suspending charcoal in cellophane bags, in dialyzing thimbles, and in masses of agar, and so forth, and placing each, in turn, in Cohen-Wheeler broth. Growth of *H. pertussis* increased greatly.

As an agar base we have used the pertussis fluid culture medium described by Cohen-Wheeler (*2*). To this medium we have added 2 to 3 percent agar and 0.4 percent charcoal (Norite sg.). The complete medium, quite black in color, is put into large test tubes to make slants for phase I stock cultures, and into "toxin bottles" (New York State Department of Health pyrex bottles used commonly for diphtheria toxin preparation) or any other desirable bottles for growing mass cultures. The tubes and bottles are subjected to ordinary autoclave sterilization for one-half hour. Length of autoclaving will generally depend on the size and number of bottles loaded into the autoclave and also on the size of the autoclave. For these reasons, fixed rules for autoclaving can hardly be given except to state that minimum heating to effect sterilization is preferable.

We regulate the temperature for incubating cultures to 35° to 36° C. There is some slight indication that this level is preferable to 37° to 38° C. Some variation in temperature is usual in most large incubators according to our experience and the personal inquiries we have made.

H. pertussis grows luxuriantly on charcoal agar, and in gram-stained smears the organisms are quite small gram-negative rods in

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24- or 48-hour cultures. Saline suspensions of such cultures agglutinate to high titer (1:5000 to 1:10,000) in phase I rabbit antiserum, and positive Dold reactions are produced in rabbit skin. Cultures of *H. pertussis* on charcoal agar fulfill the criteria for S forms as indicated by Shibley and Hoelscher (*3*) except insofar as charcoal may substitute for blood in the culture medium previously specified by definition for growing S forms.

Freshly isolated cultures of *H. pertussis*, or vaccine strains of proved antigenicity under the customary dehydration, which are cultivated on charcoal agar, produce, in most instances, vaccine of acceptable potency when assayed by the current National Institutes of Health (N.I.H.) mouse immunity and toxicity tests. We have subcultured some of these strains for as many as 15 or 20 generations on charcoal agar slants, and, at this time, have prepared vaccine on charcoal agar bottles. This vaccine, in most cases, passes the N.I.H. test. Some occasional strains of *H. pertussis* which have been unsatisfactory vaccine producers in the Cohen-Wheeler broth have promptly produced satisfactory vaccine when cultured on charcoal agar.

Of the *H. pertussis* cultures used, 40103 and 41405 were supplied to us by Dr. Harold Lyall of the New York State Department of Health Laboratories; 3722, 3734, and 3739 were Lilly Laboratory strains originally obtained from Dr. Pearl Kendrick; W7, Cooper, Corbin, Newton, and Rainey were newly isolated strains from Dr. Dwain Walcher.

Listed in the table are the results of N.I.H. mouse immunization tests using the standard methods with 42 lots of vaccine made with 10 different cultures. Several repeat lots of vaccine were made with the same seed culture generation to test repeatability of results. Of the 42 lots, 37 were equal to or better than the reference vaccine: 5 lots, marked by parentheses, were inferior to the reference vaccine; however, 3 of the 5 were close to the potency of the reference vaccine, while 2 were very inferior for reasons not known. Basis for comparing these vaccines is their ED_{50} value which is the amount of vaccine necessary to confer immunity on 50 percent of the mice challenged by intracerebral injection of 100,000 *H. pertussis*, the LD_{50} of which is 1,000 organisms or less.

Seven lots of the charcoal agar vaccine, marked in the table, were very much better than the reference vaccine. However, precise computation was not possible since the results fell outside the optimum limits in relation to vaccine dosage, and so forth. In one case, the same was true of the N.I.H. reference vaccine (see table).

This brief report has been prepared because the growth of phase I strains on charcoal agar appears so luxuriant and suffices as potent vaccine. Furthermore, a good many subcultures may be made on charcoal agar without degrading the strain as a good vaccine producer.

Results of N.I.H. Mouse Immunization Tests

Lot No. of vaccine	Vaccine made from charcoal agar subculture number:	*H. pertussis* strain	ED_{50} of vaccine (x 1 million)	ED_{50} of reference vaccine N.I.H. No. 4 (x 1 million)
921	10	W7	94	472
883	6	3722	162	268
887	7	3722	303	463
881	5	3734	[1] 33	[2] 81
923	10	3734	195	472
939	20	3734	92	383
847	5	3739	277	488
893	[3] 5	3739	331	752
873	6	3739	[1] 51	173
953	[3] 6	3739	(415)	383
849	7	3739	381	463
851	[3] 7	3739	61	173
897	8	3739	[1] 33	240
899	[3] 8	3739	[1] 88	240
853	9	3739	207	240
855	[3] 9	3739	(720)	240
919	10	3739	(218)	197
857	11	3739	157	463
859	[3] 11	3739	[1] 33	463
861	13	3739	194	240
863	[3] 13	3739	126	240
865	15	3739	[1] 43	240
867	[3] 15	3739	[1] 176	488
801	5	3739 and 41405	239	300
813	[3] 5	3739 and 41405	544	574
819	[3] 5	3739 and 41405	185	221
819A	[3] 5	3739 and 41405	217	238
821	[3] 5	3739 and 41405	132	238
823	6	3739 and 41405	123	224
837	[3] 6	3739 and 41405	228	240
839	[3] 6	3739 and 41405	185	259
891	8	40103	272	463
843	5	Cooper	90	259
947	6	do	206	472
927	10	do	128	472
943	20	do	(530)	236
907	4	Corbin	191	240
959	7	do	100	472
925	10	do	57	197
941	20	do	160	472
909	4	Newton	(290)	240
977	4	Rainey	200	472

[1] Much superior to reference vaccine.
[2] Results outside the optimum limits of vaccine dosage.
[3] Repeat lots of vaccine made.

A few tests not reported here appear to indicate that the Cohen-Wheeler base for the charcoal agar medium may be simplified somewhat since the medium ingredients are not included in the vaccine as is the case with broth.

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Incidence of Disease

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

Reports From States for Week Ended February 24, 1951

Measles. The total number of new cases of measles reported for the current week was 14,918 which is 80 percent greater than the number reported for the same week last year. The States reporting the largest number of cases for the current week were Texas, 2,968; California, 1,637; Colorado, 801; Wisconsin, 736; and Pennsylvania, 634.

Influenza. The total number of cases of influenza reported for the current week was 6,149, as compared with 6,007 for the same week last year and 4,204 for a 5-year median. California reported 107 cases for the current week, but official reports indicate that the incidence is subsiding in previously reported areas. However, mild respiratory infections are prevalent in some other parts of the State. There are no official reports of epidemics of influenza occurring presently in areas other than California and the northeastern section of the country in which the diagnosis has been confirmed by isolation of the influenza virus or by serological tests. The total number of deaths reported in 106 large cities of the country, although slightly above the median, does not indicate any significant increase which might be attributed to influenza. The cities in the Middle Atlantic States which showed an increase of 7 percent in the number of deaths for the week ended February 17, increased only 0.5 percent during the current week.

NIH Influenza Information Center

The regional laboratory at the California Department of Public Health reports that 109 paired serum specimens have been examined for influenza from January 1 to February 16, 1951. Of these, 35 showed a significant rise in titer by the complement fixation test. In those tested by the hemagglutination inhibition test, the infecting virus was identified as belonging to the A-prime subgroup.

Dr. George K. Hirst of the Public Health Research Institute of New York City, reports the isolation of nine strains from throat washings taken on February 17 from mild cases of influenza occurring in the Bronx Veterans' Hospital. Three of these strains have been typed and were found to be closely related to the FM–1 strain.

Dr. A. S. Lazarus of the University of Washington, Seattle, reports that paired serum specimens from a single patient at the University infirmary showed a diagnostically significant increase in titer for influenza A-prime by both complement fixation and hemagglutination inhibition tests. This is the first identification of influenza reported in this area this winter, but there is no evidence of widespread distribution of the disease on the campus.

The Department of Virus and Rickettsial Diseases of the Army Medical Service Graduate School reports the isolation of a strain of virus of the A-prime group which, with rooster antiserum, resembles the Cuppett strain. This was recovered from a member of the armed forces stationed in Greenland. Of seven paired sera from an Air Force base in Labrador, six were positive in the hemagglutination inhibition test for the general A group.

The Division of Preventive Medicine, Office of the Surgeon General of the Air Force, reports that one paired serum from an Air Force base in New Mexico showed rise in hemagglutination inhibition titer for A and A-prime.

The Regional Laboratory at the Boston City Hospital (Dr. Maxwell Finland) reports isolation of four strains of virus from recent cases. Two of these, from a laboratory worker and from an interne, resembled the PR–8 strain when tested with rabbit antisera. They were found to infect the allantoic cavity of chick embryo on primary passage. Two other strains isolated from patients in the hospital resembled the FM–1 strain.

Comparative Data for Cases of Specified Reportable Diseases: United States

[Numbers after diseases are International List numbers, 1948 revision]

Disease	Total for week ended—		5-year median 1946–50	Seasonal low week	Cumulative total since seasonal low week		5-year median 1945–46 through 1949–50	Cumulative total for calendar year—		5-year median 1946–50
	Feb. 24, 1951	Feb. 25, 1950			1950–51	1949–50		1951	1950	
Anthrax (062)	3		2	(1)	(1)	(1)	(1)	13	2	11
Diphtheria (055)	96	150	211	27th	[2] 3,684	5,617	8,171	[2] 777	1,346	1,813
Encephalitis, acute infectious (082)	14	16	7	(1)	(1)	(1)	(1)	86	89	61
Influenza (480–483)	6,149	6,007	4,204	30th	40,359	36,751	36,751	25,817	26,167	26,167
Measles (085)	14,918	8,172	15,725	35th	114,097	64,131	95,323	85,396	45,001	69,199
Meningitis, meningococcal (057.0)	115	85	85	37th	1,820	1,609	1,609	859	696	696
Pneumonia (490–493)	2,019	2,457		(1)	(1)	(1)	(1)	14,336	18,659	
Poliomyelitis, acute (080)	90	96	40	11th	33,216	42,365	25,284	997	891	487
Rocky Mountain spotted fever (104)				(1)	(1)	(1)	(1)	2	6	5
Scarlet fever (050)[3]	2,365	1,862	2,868	32d	33,429	30,277	44,553	17,738	13,838	20,705
Smallpox (084)		1	3	35th	14	19	46	6	10	25
Tularemia (059)	9	20	21	(1)	(1)	(1)	(1)	114	186	186
Typhoid and paratyphoid fever (040,041)[4]	31	46	40	11th	3,220	3,736	3,736	305	363	333
Whooping cough (056)	1,604	2,447	2,251	39th	34,715	41,034	41,034	13,113	19,498	17,994

[1] Not computed. [2] Deduction: Mississippi, week ended Jan. 27, 1 case. [3] Including cases reported as streptococcal sore throat. [4] Including cases reported as salmonellosis.

Reported Cases of Selected Communicable Diseases: United States, Week Ended Feb. 24, 1951

[Numbers under diseases are International List numbers, 1948 revision]

Area	Diphtheria (055)	Encephalitis, infectious (082)	Influenza (480–483)	Measles (085)	Meningitis, meningococcal (057.0)	Pneumonia (490–493)	Poliomyelitis (080)
United States	**96**	**14**	**6,149**	**14,918**	**115**	**2,019**	**90**
New England	**1**		**301**	**822**	**3**	**84**	
Maine			164	154		15	
New Hampshire			40	176		5	
Vermont			60	139			
Massachusetts	1			266	3		
Rhode Island			2	1			
Connecticut			35	86		64	
Middle Atlantic	**4**	**2**	**77**	**1,673**	**17**	**360**	**5**
New York	1	1	[1] 49	516	7	111	5
New Jersey	1	1	28	523	4	125	
Pennsylvania	2			634	6	124	
East North Central	**8**	**5**	**54**	**2,308**	**16**	**185**	**5**
Ohio	2			434	4		
Indiana	2			113	1	5	
Illinois	3	2	11	589	6	106	1
Michigan	1	3	43	436	2	74	3
Wisconsin				736	3		1
West North Central	**6**		**19**	**975**	**6**	**39**	**9**
Minnesota	3		2	92	1	4	
Iowa				42	4		3
Missouri	2		2	418	1		1
North Dakota			8	96		22	
South Dakota	1			4			3
Nebraska			4	12			1
Kansas			3	311		13	1
South Atlantic	**29**		**1,773**	**1,514**	**20**	**242**	**15**
Delaware				18			
Maryland	1		2	67	1	50	
District of Columbia			1	48		6	1
Virginia			755	309	5	132	
West Virginia	2		327	277		19	3
North Carolina	12			233	4		4
South Carolina	10		348	13	7	11	
Georgia	4		340	470	1	24	2
Florida				79	2		5
East South Central	**19**	**2**	**1,344**	**452**	**16**	**42**	**6**
Kentucky	1		4	174	4	7	2
Tennessee	6		91	65	3		
Alabama	8	2	1,201	45	8		2
Mississippi	4		48	168	1	35	2
West South Central	**22**		**1,458**	**3,685**	**23**	**873**	**16**
Arkansas	2		441	314		74	
Louisiana	1		896	156	1	61	2
Oklahoma	3		121	247	6	37	1
Texas	16			2,968	16	701	13
Mountain	**1**	**3**	**608**	**1,293**	**1**	**93**	**12**
Montana			29	54		6	
Idaho				42			2
Wyoming		2		43		7	1
Colorado			30	801	1	22	2
New Mexico		1		49		6	1
Arizona	1		529	272		52	6
Utah				25			
Nevada			20	7			
Pacific	**6**	**2**	**515**	**2,196**	**13**	**101**	**22**
Washington			140	542	3	2	3
Oregon	1		268	17		32	4
California	5	2	107	1,637	10	67	15
Alaska							
Hawaii			12				

[1] New York City only.

Anthrax: Pennsylvania, 2 cases; New Jersey, 1 case.

Reported Cases of Selected Communicable Diseases: United States, Week Ended Feb. 24, 1951—Continued

[Numbers under diseases are International List numbers, 1948 revision]

Area	Rocky Mountain spotted fever (104)	Scarlet fever (050)	Smallpox (084)	Tularemia (059)	Typhoid and paratyphoid fever [1] (040, 041)	Whooping cough (056)	Rabies in animals
United States		**2,365**		**9**	**31**	**1,604**	**114**
New England		**209**				**158**	
Maine		20				48	
New Hampshire		8				8	
Vermont		2				24	
Massachusetts		132				51	
Rhode Island		9				19	
Connecticut		38				8	
Middle Atlantic		**387**			**5**	**238**	**18**
New York		[2] 189				74	18
New Jersey		68			1	70	
Pennsylvania		130			4	94	
East North Central		**672**		**1**	**6**	**235**	**4**
Ohio		142			1	52	1
Indiana		78			4	21	
Illinois		124		1		19	1
Michigan		276			1	82	2
Wisconsin		52				61	
West North Central		**137**		**1**		**83**	**8**
Minnesota		35				18	1
Iowa		21				8	6
Missouri		30		1		6	
North Dakota		2				13	
South Dakota		5				9	
Nebraska		11				2	
Kansas		33				27	1
South Atlantic		**217**		**1**	**6**	**264**	**26**
Delaware		7					
Maryland		22				11	
District of Columbia		12			2	10	
Virginia		30				84	6
West Virginia		5			1	66	7
North Carolina		94			1	42	
South Carolina		12			1	4	8
Georgia		15			1	24	4
Florida		[3] 20		1		23	1
East South Central		**73**		**3**	**2**	**42**	**36**
Kentucky		20			1	23	12
Tennessee		41			1	5	13
Alabama		6		2		11	11
Mississippi		6		1		3	
West South Central		**84**		**3**	**5**	**405**	**22**
Arkansas		4		2	2	78	3
Louisiana		17		1		5	
Oklahoma		9				23	
Texas		54			3	299	19
Mountain		**234**			**1**	**137**	
Montana		8				6	
Idaho		86				8	
Wyoming						4	
Colorado		27				18	
New Mexico		5			1	55	
Arizona		7				43	
Utah		[3] 101				3	
Nevada							
Pacific		**352**			**6**	**42**	
Washington		93				8	
Oregon		50				5	
California		[3] 209			6	29	
Alaska							
Hawaii		5					

[1] Including cases reported as salmonellosis.
[2] Including cases reported as streptococcal sore throat.

FOREIGN REPORTS

CANADA

Reported Cases of Certain Diseases—Week Ended Feb. 10, 1951

Disease	Total	Newfoundland	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia
Brucellosis	2							1			1
Chickenpox	1,441			57	1	221	766	43	68	141	144
Diphtheria	3					2	1				
Dysentery, bacillary	7					4		1			2
German measles	336			19		31	180	1	12	44	49
Influenza	6,300	476		2,710	46	554	51	5	286		2,172
Measles	2,575	5		43	5	232	2,048	145	23	26	48
Meningitis, meningococcal	1										1
Mumps	1,496	3		21	1	180	539	37	151	317	247
Poliomyelitis	1							1			
Scarlet fever	312			5		51	49	18	22	91	76
Tuberculosis (all forms)	172	8		4	6	42	20	14	3	32	43
Typhoid and paratyphoid fever	9					3	1			5	
Venereal diseases:											
Gonorrhea	274	6		6	3	74	39	21	6	30	89
Syphilis	128	7		13	8	46	18	9	9	3	15
Primary	6					2	2		1		1
Secondary	4					1			2	1	
Other	118	7		13	8	43	16	9	6	2	14
Other forms	2										2
Whooping cough	219				32	41	82	8	4	3	49

REPORTS OF CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER RECEIVED DURING THE CURRENT WEEK

The following reports include only items of unusual incidence or special interest and the occurence of these diseases, except yellow fever, in localities which had not recently reported cases. All reports of yellow fever are published currently. A table showing the accumulated figures for these diseases for the year to date is published in the PUBLIC HEALTH REPORTS for the last Friday in each month.

Cholera

India. During the week ended February 17, 1951, 96 cases of cholera were reported in Calcutta, compared with 82 and 71 for the weeks ended February 3 and 10, respectively. For the week ended February 17, Negapatam reported 10 cases and Madras reported 7.

India (French). For the week ended February 3, 1951, cholera was reported in seaports as follows: Karikal eight cases and Pondicherry nine.

India. During the week ended February 17, 1951, 637 cases of smallpox were reported in Calcutta as compared with 430 for the

Smallpox

previous week. The figure for February 17 is larger than that for any week during 1949 and 1950. The cumulative number of cases (3,115) for the first 7 weeks of 1951 was more than twice the number (1,261) for the corresponding period in 1950. During the same period in 1949 less than 200 cases were reported. For the week ended February 17, Madras and Bombay reported 79 and 72 cases, respectively. In Nagpur the indications are that the smallpox epidemic has reached its peak since 108 cases were reported for the week ended February 10 as compared with 204 for the previous week.

India (French). During the week ended February 3, 1951, 91 cases of smallpox were reported in Pondicherry.

Pakistan. For the week ended February 17, 1951, nine cases of smallpox were reported in Karachi as compared with two for the previous week.

Yellow Fever

Brazil. The outbreak of jungle yellow fever in Goiaz State has spread to counties in the eastern and southern part of the State. The area involved is one which has been opened for settlement during the past 3 or 4 years. During this period the population increased from 10,000 to approximately 200,000. An estimated 2,000 cases (400 deaths) have occurred during the current outbreak.

Plague Infection in Lea County, N. Mex.

A report dated February 19, 1951, states that plague infection was proved positive in a specimen of 16 fleas taken from 8 harvest mice, *Onychomy leucogaster,* trapped about 5 miles east of Eunice in Lea County on January 30, 1951.

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It contains (1) current information regarding the incidence and geographic distribution of communicable diseases in the United States, insofar as data are obtainable, and of cholera, plague, smallpox, typhus fever, yellow fever, and other important communicable diseases throughout the world; (2) articles relating to the cause, prevention, and control of disease; (3) other pertinent information regarding sanitation and the conservation of the public health.

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